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DATA EVALUATION REPORT

STUDY TYPE: Reproductive Toxicity (Rat); Guideline Series 83-4

EPA IDENTIFICATION NUMBERS

TOX CHEM. NUMBER.:

MRID NUMBER: 428887-03

TEST MATERIAL: 4-Dichloroacetyl-3,4-dihydro-3-methyl-2H-1,4-benzoxazine

SYNONYMS: Benoxacor, CGA 154281 technical

SPONSOR: CIBA-GEIGY Limited, Basel, Switzerland

STUDY NUMBER: 380-154

TESTING FACILITY: Hazleton Laboratories, Deutschland GmbH, Münster, Germany

TITLE OF REPORT: Two-Generation Oral (Dietary Administration) Reproduction Toxicity Study in the Rat on CGA 154281 (One Litter per Generation)

AUTHOR: I. Osterburg

REPORT ISSUED: December 1991

CONCLUSIONS: In a two-generation reproduction study, Sprague-Dawley rats received benoxacor continuously in the diet for two successive generations at dosages of 0, 10, 50, 500, and 1000 ppm (during premating for F_1 males: 0.83, 4.20, 45.45, and 89.21 mg/kg/day, respectively; for F_1 females: 0.92, 4.57, 49.16, and 93.53, respectively).

Parental NOEL = 50 ppm
Parental LOEL = 500 ppm based on decreased body weight and/or weight gain in both sexes and generations

In addition to significantly decreased body weight and/or weight gain in parental animals of both generations, food consumption decreased significantly in F_1 females at 1000 ppm.

Reproductive NOEL = 50 ppm
Reproductive LOEL = 500 ppm based on decreased pup body weight on lactation day (LD) 21 in both generations

011508

CLASSIFICATION: Core Guideline Data. This study meets the requirements set forth under Guideline Series 83-4 for a two-generation reproductive toxicity study in rats.

A. MATERIALS

Test Compound

Purity:	96.8%
Description:	Brownish powder
Batch number:	SG 7505-11
Date received:	December 19, 1989
Contaminants:	None reported
Storage:	Room temperature

Test Animals

Species:	Rat
Strain:	Sprague-Dawley Crl: CD (SD)BR
Source:	Charles River Wiga GmbH, Sulzfeld, Germany
Age:	Approximately 6 weeks at receipt
Weight:	F_0 males--174-232 g on study day 1 F_0 females--143-175 g on study day 1

B. STUDY DESIGN

This study was designed to assess the potential of benoxacor to cause reproductive toxicity in rats following daily dietary administration for two successive generations.

Mating Procedure

After 8 days of acclimatization followed by 14 weeks of dietary treatment, F_0 females were paired with males from the same group in a ratio of 1:1 until a copulatory plug was detected or sperm was observed in a vaginal smear (or for a maximum of 21 days). All females that did not mate within the first 14 days were paired with proven males from the same dosage group for an additional 7 days. The day on which mating was confirmed was considered gestational day (GD) 0. F_1 animals were mated in a similar manner avoiding sibling pairs.

Animal Husbandry

Rodent diet (Ssniff R 10) and tap water were available ad libitum. Temperature and humidity were maintained at 19-25°C and 30%-70%, respectively. A 12-hour light/dark cycle was maintained. The study authors did not report the number of air changes per hour.

Group Arrangement

The F_0 animals were divided into groups using a stratified randomization procedure based on body weight. F_1 animals were stratified based on randomly drawn cards. The groups were as follows:

Test Group	Target Conc. (ppm)	Number of Animals Assigned per Group			
		F_0		F_1	
		Males	Females	Males	Females
Control	0	25	25	25	25
Low dose	10	25	25	25	25
Low-mid dose	50	25	25	25	25
High-mid dose	500	25	25	25	25
High dose	1000	25	25	25	25

Dosage Administered

To achieve homogenous mixtures, the test material particle size was standardized before it could be mixed with the diet in several steps. These steps were not described by the study author. Test diets were prepared monthly and stored at room temperature except for diets from the low-dosage group which were stored at -20°C. Diets were not adjusted for active ingredient. Analyses for concentration and homogeneity were conducted 16 times throughout the study. Analyses for stability were conducted three times during the current study and also in a previous study (HLD project no. 380-183).

Dosage Rationale

Dosages were selected based upon the results of a range-finding study (HLD Project No. 380-153). The results of this study were not presented.

Observations

Observations were made twice daily for mortality and moribundity and at least once daily for clinical signs of toxicity. Body weight data were recorded weekly during premating and mating. Body weight data for females were also recorded on GDs 0, 7, 14, and 20 and on LDs 1, 4, 7, 14, and 21. Food consumption data were recorded twice weekly during premating for both males and females and at 1-6-day intervals for females during gestation and lactation.

The following data were recorded for each litter:

- Number of live and dead pups, sex, external anomalies, and individual pup weight on LDs 1, 4, 7, 14, and 21
- Abnormalities of nesting or nursing behavior
- Fetal development including time of pinna unfolding, incisor eruption, and eye opening
- Pupillary reflex and auditory response for two pups/sex/litter on LD 21

Uteri from apparently nonpregnant females were stained with a 10% ammonium sulfide solution to detect early embryonic loss.

On day 4, pups were randomly culled to four/sex/litter whenever possible. Pups dying or killed during lactation were examined for external and visceral abnormalities. Twenty-five male and 25 female F_1 pups were randomly selected as F_1 parental animals. All remaining F_1 and F_2 pups were killed after weaning and necropsied.

Parental males and females were sacrificed and necropsied after weaning of their respective litters on approximately LD 26. The following tissues were preserved in 10% neutral buffered formalin and processed for histological examination from animals in the control and high-dosage groups. All organs marked with an asterisk (*) were also weighed.

Pituitary	Ovaries
Bile duct	*Testes
Stomach	*Epididymides
*Liver	Seminal vesicles
*Kidneys	Coagulating gland
Vagina	Prostate
Uterus	Gross lesions
Cervix	

Statistical Analysis

The following analyses were conducted:

- Parental body weight, weight gain, and food consumption--Levene's test, ANOVA, and Dunnett's test

- Mating performance; gestation length; pup body weight; numbers of implantations, live fetuses, and dead fetuses; live birth, viability, and weaning indices; sex ratio; pup development parameters; litter weights; and organ weights--ANOVA with one factor treatment and Student-Newman-Keuls test

Compliance

The following statements were provided:

- A signed Statement of No Data Confidentiality Claims, dated July 26, 1993

- A signed Statement of Compliance with EPA, OECD, Canada Health Protection Branch, and Japanese MAFF GLPs, dated July 5, 1993

- A signed Quality Assurance Statement, dated December 2, 1991

C. RESULTS

Test Material Analysis

Analyses for concentration and homogeneity of the test material at all dosage levels in the diet revealed mean values that were 69%-128% of target. Analysis for stability revealed values that were on average 74%, 77%, and 91% of target after 28 days at room temperature for diets prepared at 50, 500, and 1000 ppm, respectively, and 77% after 28 days deep frozen for diets prepared at 10 ppm.

Parental Toxicity

Mortality: No compound-related mortalities were observed at any dosage level in either sex or generation. In the F_0 generation, one female at 500 ppm died during delivery. Necropsy revealed kidney changes.

In the F_1 generation, one female at 10 ppm was found dead on day 58 of the study. Necropsy revealed changes in the lungs, liver, esophagus, kidneys, and stomach. In addition, one female at 500 ppm was found dead on day 5 of the study. Because of severe autolysis, a necropsy was not performed on this animal.

Clinical observations: No compound-related clinical signs were observed at any dosage level in either sex or generation.

Body weight: Compound-related effects on body weight and/or weight gain were observed in a dose-dependent manner at 500 and/or 1000 ppm in both sexes and generations. Summaries of body weight gain data for selected intervals are presented in Tables 1, 2, and 3. Detailed results are discussed below.

In males of the F_0 generation, body weight (data not shown) decreased significantly from day 22 of premating through the end of the mating period at 1000 ppm (92%-94%) and on premating day 99 at 500 ppm (94%). It increased significantly on day 15 at 500 ppm (107%). Body weight gain (Table 1) decreased significantly during premating on days 1-8, 15-22, and 1-99 at 1000 ppm and on days 15-22, 43-50, and 1-99 at 500 ppm. It increased significantly on days 8-15 at 500 ppm. The decreased body weight at 1000 ppm and decreased body weight gain on days 1-99 at 1000 ppm (90%) and 500 ppm (87%) were considered to be treatment related.

In females of the F_0 generation, body weight (data not shown) decreased significantly from premating day 22 through the end of the premating period, on GDs 0, 7, and 20, and on LDs 1 and 4 at 1000 ppm (91%-95%) and on premating days 22 and 92 at 500 ppm (94%-95%). Body weight gain (Table 1) decreased significantly during premating on days 1-8, 15-22, 36-43, and 1-99 at 1000 ppm and on days 15-22 and 1-99 at 500 ppm. It increased significantly on premating days 22-29 at 500 ppm and LD 1-21 at 1000 ppm. The decreased body weight at 1000 ppm and decreased body weight gain on days 1-99 at 1000 ppm (90%) and 500 ppm (82%) were considered to be treatment related.

TABLE 1. Body Weight Gain (g ± S.D.) During the Premating Period for Rats Receiving Benoxacor for Two Successive Generations[a]

Days of Treatment	Observation at Each Exposure Level (ppm)				
	0	10	50	500	1000
F_0 Males					
1- 8	56.4 ± 7.8	59.5 ± 7.7	57.1 ± 9.7	51.9 ± 10.6	49.0 ± 8.3*
15-22	36.2 ± 5.8	29.1 ± 7.5**	31.9 ± 6.0	6.1 ± 10.5**	26.3 ± 8.2**
36-43	21.7 ± 7.0	15.7 ± 10.1*	20.6 ± 7.4	21.0 ± 7.5	16.9 ± 10.2
64-71	9.9 ± 4.9	10.2 ± 6.6	12.4 ± 6.1	9.4 ± 11.7	9.5 ± 5.6
92-99	9.8 ± 4.0	5.9 ± 5.4	7.1 ± 7.1	7.0 ± 16.1	3.2 ± 15.6
1-99	307.9 ± 32.5	292.5 ± 38.0	298.3 ± 37.5	277.7 ± 39.4*	268.0 ± 49.9**
F_0 Females					
1- 8	22.5 ± 4.9	22.2 ± 5.2	23.2 ± 3.9	19.7 ± 5.4	18.6 ± 4.3*
15-22	17.4 ± 5.9	14.6 ± 5.3	14.8 ± 3.8	10.9 ± 4.8**	8.7 ± 5.1**
36-43	8.0 ± 4.9	5.6 ± 4.9	7.2 ± 5.8	6.7 ± 5.3	3.6 ± 5.5*
64-71	4.0 ± 5.0	2.9 ± 4.4	3.2 ± 4.8	5.3 ± 13.4	1.9 ± 3.9
92-99	1.2 ± 5.2	0.7 ± 7.5	1.8 ± 5.5	2.5 ± 7.1	3.6 ± 5.6
1-99	115.2 ± 13.9	109.5 ± 17.3	108.6 ± 12.4	104.2 ± 16.0*	94.7 ± 12.7**
F_1 Males					
1- 8	65.2 ± 11.4	58.6 ± 9.3	61.4 ± 8.3	55.8 ± 10.3**	53.0 ± 8.4**
15-22	51.9 ± 13.0	55.7 ± 5.9	47.7 ± 15.8	49.3 ± 9.2	43.0 ± 13.4**
36-43	29.9 ± 11.9	32.4 ± 7.5	29.9 ± 5.8	27.7 ± 8.5	25.0 ± 11.5
64-71	13.6 ± 7.5	15.6 ± 6.2	16.0 ± 6.1	12.6 ± 6.4	15.5 ± 4.3
92-99	7.7 ± 5.6	4.2 ± 4.3	6.0 ± 3.8	4.0 ± 10.0*	4.6 ± 7.9
1-99	401.6 ± 48.8	404.2 ± 35.9	399.8 ± 41.6	369.5 ± 51.1	352.9 ± 59.1**
F_1 Females					
1- 8	50.6 ± 7.4	46.0 ± 9.4	42.7 ± 6.2**	43.1 ± 8.5**	40.0 ± 7.6**
15-22	23.2 ± 5.6	23.8 ± 6.1	22.5 ± 4.7	22.8 ± 5.3	22.5 ± 4.6
36-43	9.7 ± 5.5	9.0 ± 5.2	13.1 ± 7.2	11.0 ± 5.1	11.3 ± 8.7
64-71	3.7 ± 4.4	4.8 ± 5.0	3.7 ± 4.4	5.6 ± 4.0	5.0 ± 3.6
92-99	-1.4 ± 5.6	2.3 ± 5.6	2.1 ± 4.3	3.7 ± 3.5**	3.3 ± 3.4**
1-99	193.6 ± 19.0	190.1 ± 23.1	182.5 ± 18.9	179.5 ± 26.3	168.8 ± 20.3**

[a]Data were extracted from Study No. 380-154, Tables 4 and 29.

*Significantly different from control ($p \leq 0.05$)

**Significantly different from control ($p \leq 0.01$)

TABLE 2. Body Weight Gain (g ± S.D.) During Gestation for Rats Receiving Benoxacor for Two Successive Generations[a]

Gestation Day	Observation at Each Exposure Level (ppm)				
	0	10	50	500	1000
F_0 Generation - F_1 litters					
0- 7	18.3 ± 9.2	21.1 ± 4.9	22.1 ± 9.8	23.2 ± 7.2	23.4 ± 8.4
7-14	20.2 ± 7.6	21.4 ± 5.8	20.5 ± 10.6	20.2 ± 4.6	22.2 ± 4.6
14-20	67.8 ± 9.7	69.0 ± 13.2	61.9 ± 16.8	64.8 ± 10.5	63.3 ± 8.7
0-20	106.2 ± 16.1	111.5 ± 15.5	104.5 ± 20.9	108.3 ± 14.2	108.9 ± 14.6
F_1 Generation - F_2 litters					
0- 7	22.7 ± 6.8	20.2 ± 8.2	17.3 ± 6.8*	18.0 ± 5.8	18.7 ± 4.8
7-14	24.2 ± 6.0	25.3 ± 4.6	24.3 ± 4.5	22.9 ± 5.6	22.6 ± 5.5
14-20	70.1 ± 14.2	68.6 ± 9.6	68.9 ± 10.7	64.2 ± 8.6	58.4 ± 7.9**
0-20	117.0 ± 17.2	114.1 ± 14.2	110.5 ± 12.9	105.1 ± 14.2*	99.6 ± 9.6**

[a]Data were extracted from Study No. 380-154, Tables 6 and 31.

*Significantly different from control ($p \leq 0.05$)

**Significantly different from control ($p \leq 0.01$)

TABLE 3. Body Weight Gain (g ± S.D.) During Lactation for Rats Receiving Benoxacor for Two Successive Generations[a]

Lactation Day	Observation at Each Exposure Level (ppm)				
	0	10	50	500	1000
F_0 Generation - F_1 litters					
1- 4	9.1 ± 14.7	10.6 ± 11.6	6.9 ± 12.8	6.8 ± 9.3	12.9 ± 8.7
4- 7	2.6 ± 15.2	3.4 ± 9.4	4.5 ± 10.8	2.1 ± 10.0	8.5 ± 8.2
7-14	14.7 ± 19.0	15.0 ± 16.4	5.3 ± 18.6	9.7 ± 16.5	16.1 ± 13.2
14-21	-1.8 ± 17.1	1.6 ± 13.8	3.8 ± 17.3	6.4 ± 18.1	2.6 ± 10.9
1-21	24.7 ± 18.5	30.6 ± 21.1	19.7 ± 22.5	25.8 ± 20.6	40.1 ± 18.8*
F_1 Generation - F_2 litters					
1- 4	20.9 ± 10.8	19.0 ± 10.1	13.1 ± 9.6	14.6 ± 6.7	14.0 ± 8.3
4- 7	2.8 ± 8.4	9.4 ± 10.6	6.1 ± 9.1	10.4 ± 8.0*	9.0 ± 9.1
7-14	13.5 ± 11.1	11.5 ± 16.0	16.4 ± 10.6	13.1 ± 6.0	14.7 ± 8.4
14-21	-8.8 ± 13.3	3.0 ± 13.5**	-4.9 ± 12.1	-1.0 ± 8.6	3.2 ± 8.7**
1-21	28.4 ± 16.3	42.9 ± 17.6**	30.7 ± 14.7	37.0 ± 14.9	40.9 ± 15.1*

[a]Data were extracted from Study No. 380-154, Tables 8 and 33.

*Significantly different from control ($p \leq 0.05$)

**Significantly different from control ($p \leq 0.01$)

In males of the F_1 generation, body weight (data not shown) decreased significantly from premating day 8 through the end of the mating period at 1000 ppm (87%-89%) and at 500 ppm (86%-93%). Body weight gain (Table 1) decreased significantly during premating on days 1-8, 15-22, 71-78, and 1-99 at 1000 ppm and on days 1-8 and 92-99 at 500 ppm. The decreased body weight at 1000 and 500 ppm and decreased body weight gain on days 1-99 at 1000 ppm (88%) were considered to be treatment related.

In females of the F_1 generation, body weight (data not shown) decreased significantly throughout the premating period at 1000 ppm (82%-86%) and from day 8 through the end of the premating period at 10 ppm (85%-94%), 50 ppm (91%-95%), and 500 ppm (87%-91%). During gestation, body weight decreased significantly on days 0, 7, 14, and 20 at 1000 ppm (87%) and 500 ppm (91%); on days 7, 14, and 20 at 50 ppm (93%-95%); and on day 7 at 10 ppm (94%). Body weight gain (Tables 1) decreased significantly at 1000 ppm on premating days 1-15, 29-36, 43-50, 57-64, 78-85, and 1-99 at 1000 ppm; on premating days 1-8, 29-36, 57-64, 78-85, and 1-99 at 500 ppm; and on premating days 1-8, 29-36, 43-50, and 57-64 at 50 ppm. During gestation (Table 2), body weight gain decreased significantly on days 14-20 and 0-20 at 1000 ppm, on days 0-20 at 500 ppm, and on days 0-7 at 50 ppm. During lactation (Table 3), body weight gain increased significantly on days 14-21 and 1-21 at 10 and 1000 ppm and on days 4-7 at 500 ppm. The decreased body weight at 1000 and 500 ppm and decreased body weight gain on premating days 1-99 at 1000 ppm (87%) and on GDs 0-20 at 1000 ppm (90%) and 500 ppm (85%) were considered to be treatment related. The decreased body weight at 10 and 50 ppm was not considered to be toxicologically relevant since it did not influence the body weight gain.

<u>Food consumption</u>: Compound-related effects on food consumption (g/animal/day) were observed at 1000 ppm in F_1 females. Summaries of food consumption data for selected intervals are presented in Tables 4 and 5. Detailed results are discussed below.

In males of the F_0 generation (Table 4), food consumption deviated (increased or decreased) significantly from control during premating at 1000 ppm on days 15-19, 40-43, and 75-78 and at 500 ppm on days 12-19. These deviations were not considered to be treatment related.

In females of the F_0 generation (Table 4), food consumption deviated significantly from control at 10, 50, and 1000 ppm on premating days 22-26 and at 1000 ppm on premating days 85-89 and GDs 17-20. These deviations were not considered to be treatment related.

In males of the F_1 generation (Table 4), food consumption deviated significantly from control during premating at 1000 ppm on days 1-5, 8-12, 40-43, 68-71, 85-92, and 96-99; at 500 ppm on days 1-5, 8-12, 15-19, 54-57, and 89-92; and at 50 ppm on days 8-12. These deviations were not considered to be treatment related.

In females of the F_1 generation (Table 4), food consumption decreased significantly below control during premating at 1000 ppm on days 5-8, 12-19, 22-26, 29-40, 43-50, 57-89, 92-96, and 1-99; at 500 ppm on days 12-15 and 33-36; at 50 ppm on days 33-36, 43-47, 57-61, 71-75, and

TABLE 4. Food Consumption (g/animal/day) During the Premating Period for Rats Receiving Benoxacor for Two Successive Generations[a]

Days of Treatment	Observation at Each Exposure Level (ppm)				
	0	10	50	500	1000
F_0 Males					
1- 5	24.7 ± 3.1	24.9 ± 3.2	24.9 ± 2.6	24.5 ± 4.6	23.2 ± 2.4
15-19	28.3 ± 3.8	27.7 ± 2.7	28.6 ± 2.7	33.6 ± 3.8**	26.0 ± 2.9**
29-33	27.2 ± 2.2	26.3 ± 2.3	30.0 ± 11.4	26.4 ± 2.8	25.2 ± 3.0
40-43	28.3 ± 2.9	26.9 ± 4.6	28.8 ± 3.2	29.0 ± 3.2	25.1 ± 3.6**
75-78	28.1 ± 2.5	28.7 ± 3.1	28.6 ± 2.4	28.0 ± 3.4	26.0 ± 2.9*
1-99	27.1 ± 1.9	27.2 ± 2.7	28.4 ± 2.5	27.0 ± 1.6	26.1 ± 2.6
F_0 Females					
1- 5	17.6 ± 1.6	17.7 ± 1.7	17.8 ± 2.6	17.1 ± 1.6	17.4 ± 2.7
15-19	19.5 ± 1.8	19.3 ± 1.6	19.9 ± 2.5	18.6 ± 1.8	19.1 ± 2.0
29-33	18.6 ± 1.8	18.5 ± 1.6	18.8 ± 1.8	18.3 ± 1.5	17.8 ± 1.5
40-43	21.0 ± 2.6	19.7 ± 3.0	20.8 ± 3.0	20.1 ± 2.9	18.9 ± 2.3
75-78	20.2 ± 1.9	19.9 ± 2.2	20.9 ± 3.9	19.8 ± 3.1	18.8 ± 2.3
1-99	19.3 ± 1.1	19.0 ± 1.5	19.4 ± 2.0	18.9 ± 1.5	18.5 ± 1.2
F_1 Males					
1- 5	17.8 ± 2.7	16.7 ± 2.4	16.9 ± 2.3	15.4 ± 2.7**	14.8 ± 2.6**
15-19	24.8 ± 4.4	27.5 ± 4.4	26.9 ± 7.2	33.9 ± 6.5**	27.8 ± 5.9
29-33	27.1 ± 4.8	27.0 ± 3.7	25.4 ± 3.2	25.8 ± 4.5	25.5 ± 2.5
40-43	29.1 ± 4.4	29.2 ± 3.8	28.7 ± 2.8	27.5 ± 4.6	26.1 ± 3.4*
75-78	28.9 ± 2.4	29.7 ± 3.7	29.5 ± 3.3	30.2 ± 4.0	27.8 ± 3.5
1-99	27.5 ± 2.8	27.4 ± 1.9	27.7 ± 2.6	28.3 ± 2.7	26.6 ± 1.9
F_1 Females					
1- 5	16.0 ± 1.8	14.5 ± 1.9	15.4 ± 2.2	14.5 ± 6.0	13.9 ± 1.5
15-19	18.8 ± 1.8	18.6 ± 2.0	18.8 ± 2.3	18.7 ± 1.7	17.1 ± 1.6**
29-33	19.3 ± 2.2	18.5 ± 1.9	18.6 ± 1.9	18.7 ± 2.8*	16.9 ± 1.8**
40-43	21.1 ± 2.8	19.2 ± 2.0	21.0 ± 3.5	20.4 ± 5.0	22.1 ± 6.2
75-78	20.4 ± 2.2	19.9 ± 2.2	19.8 ± 2.7	21.6 ± 5.7	17.6 ± 1.9**
1-99	20.0 ± 1.6	19.0 ± 1.7	19.2 ± 1.9	19.4 ± 2.1	17.8 ± 1.6**

[a]Data were extracted from Study No. 380-154, Tables 9 and 34.

*Significantly different from control (p≤0.05)

**Significantly different from control (p≤0.01)

TABLE 5. Food Consumption (g/animal/day) During Gestation for Rats Receiving Benoxacor for Two Successive Generations[a]

Gestation Day	Observation at Each Exposure Level (ppm)				
	0	10	50	500	1000
F_0 Generation - F_1 litters					
0- 3	20.2 ± 5.3	20.0 ± 2.8	19.4 ± 4.4	20.9 ± 3.9	18.0 ± 2.0
3- 7	20.6 ± 2.4	21.3 ± 2.5	20.8 ± 2.9	20.7 ± 2.7	19.4 ± 1.8
10-14	22.8 ± 2.4	22.6 ± 3.2	22.1 ± 3.0	22.7 ± 2.4	21.7 ± 1.6
17-20	26.5 ± 1.9	26.8 ± 2.3	26.4 ± 2.4	26.5 ± 1.9	24.8 ± 1.4*
0-20	22.6 ± 2.2	23.0 ± 1.9	22.5 ± 2.3	22.9 ± 2.2	21.4 ± 1.3
F_1 Generation - F_2 litters					
0- 3	20.3 ± 2.4	20.6 ± 3.1	19.8 ± 2.6	20.8 ± 5.5	19.4 ± 2.7
3- 7	21.9 ± 1.9	21.4 ± 2.4	21.3 ± 3.5	20.8 ± 2.6	20.1 ± 2.2
10-14	24.4 ± 2.7	23.3 ± 2.8	23.1 ± 2.1	22.9 ± 2.4	21.4 ± 1.9**
17-20	28.2 ± 3.9	27.1 ± 2.4	27.7 ± 2.5	25.9 ± 2.6*	23.4 ± 1.7**
0-20	24.0 ± 2.1	23.1 ± 2.3	23.0 ± 2.0	22.6 ± 2.5	21.4 ± 1.5**

[a]Data were extracted from Study No. 380-154, Tables 10 and 35.

*Significantly different from control ($p \leq 0.05$)

**Significantly different from control ($p \leq 0.05$)

82-85; and at 10 ppm on days 8-15, 33-36, 43-47, 57-61, and 82-85. During gestation (Table 5), food consumption decreased significantly at 1000 ppm on days 10-14, 14-17, 17-20, and 0-20 and at 500 ppm on days 14-17 and 17-20. The decreases at 1000 ppm (73%-92%) were considered to be treatment related.

Compound intake: All values for mean compound intake (mg/kg/day) were calculated by the reviewers using the summary group mean test article intake values.

In the F_0 generation, mean compound intake during premating was 0.69, 3.55, 34.84, and 68.80 mg/kg/day for males and 0.81, 4.15, 41.21, and 82.31 mg/kg/day for females at 10, 50, 500, and 1000 ppm, respectively. For females during gestation, mean compound intake was 0.72, 3.56, 36.27, and 70.66 mg/kg/day, and during lactation, it was 1.36, 6.53, 64.02, and 133.51 mg/kg/day at 10, 50, 500, and 1000 ppm, respectively.

In the F_1 generation, mean compound intake during premating was 0.83, 4.20, 45.45, and 89.21 mg/kg/day for males and 0.92, 4.57, 49.16, and 93.53 mg/kg/day. For females during gestation, mean compound intake was 0.73, 3.67, 37.65, and 73.58 mg/kg/day, and during lactation, it was 1.5,7.47, 73.25, and 149.31 mg/kg/day at 10, 50, 500, and 1000 ppm, respectively.

Gross pathology: No compound-related gross findings were observed at any dosage level in either sex or generation.

Organ weights: No compound-related effects on absolute organ weights were observed at any dosage level in either sex or generation. Relative organ weights were not determined.

Among F_1 females, absolute kidney and liver weights decreased significantly ($p \leq 0.05$) in all dosage groups. This effect did not occur in a dosage-dependent manner; it was not accompanied by histopathology findings; it was not observed in F_0 females or males of either generation; and it occurred in all treatment groups that had significantly decreased body weight. Therefore, it was considered to be an effect of decreased body weight and not directly related to treatment.

Histopathology: No compound-related effects on histopathology were observed at any dosage level in either sex or generation.

Reproductive Toxicity

Compound-related reproductive effects were observed at 500 and 1000 ppm. Summaries of reproductive parameters are presented in Tables 6 and 7. Detailed results are discussed below.

In the F_0 generation among F_1 litters (Table 6), pup body weight decreased significantly on LD 21 at 1000 ppm (88%) and 500 ppm (89%) and on LD 14 at 1000 ppm (89%). In the F_1 generation among F_2 litters (Table 7), pup body weight decreased significantly on LD 21 at 1000 ppm (88%).

TABLE 6. Effects of Exposure to Benoxacor on F_0 Reproductive Parameters and F_1 Offspring Survival and Body Weight[a,b]

Parameter	Observation at Each Exposure Level (ppm)				
	0	10	50	500	1000
No. matings	25	25	25	25	25
Insemination index (%)[c]	100	100	100	100	96
Fecundity index (%)[d]	88	96	96	88	96
Fertility index (%)[e]	88	96	96	88	92
Gestation index (%)[f]	100	100	96	96	100
Gestation length (days)	22.0	22.0	22.0	22.0	22.0
No. females with liveborn pups	22	24	24	21	23
Total no. live pups					
Day 1	265	282	261	240	282
Day 4, precull	255	266	241	208	271
Day 21	136	145	126	113	123
Mean no. live pups/litter					
Day 1	12.0 (22)	11.8 (24)	11.3 (23)	11.4 (21)	12.3 (23)
Day 4, precull	11.6 (22)	11.1 (24)	10.5 (23)	9.9 (21)	11.8 (23)
Day 21	6.2 (22)	6.0 (24)	5.5 (23)	5.4 (21)	5.3 (23)
Live birth index (%)[g]	98	99	98	99	99
Viability index (%)[h]	96	91	92	87	96
Lactation index (%)[i]	77	79	75	72	68
Mean pup body weight (g)					
Day 1	6.3	6.2	6.2	6.1	6.0
Day 7	11.9	11.3	11.7	10.7	10.7
Day 14	24.4	23.5	23.3	22.3	21.8*
Day 21	40.9	39.6	38.4	36.3*	35.8*
Sex ratio (% males, day 1)	48	47	53	53	48

[a]Data were extracted from Study No. 380-154, Tables 1, 18, 19 and 20.

[b]Number within parentheses represents number of litters.

[c]Insemination index: No. of mated females expressed as % of no. of paired females

[d]Fecundity index: No. of pregnant females expressed as % of no. of mated females

[e]Fertility index: No. of pregnant animals expressed as % of paired animals

[f]Gestation index: No. of females delivering a live litter expressed as % of no. of pregnant females

[g]Live birth index: Percentage of pups born alive based on no. of total pups born

[h]Viability index: Percentage of pups alive on day 4 based on no. of pups alive on day 1

[i]Lactation index: Percentage of pups surviving 21 days based on no. of live pups on day 4 postcull

*Significantly different from control ($p \leq 0.05$)

TABLE 7. Effects of Exposure to Benoxacor on F_1 Reproductive Parameters and F_2 Offspring Survival and Body Weight[a,b]

Parameter	Observation at Each Exposure Level (ppm)				
	0	10	50	500	1000
No. matings	25	24	25	24	25
Insemination index (%)[c]	100	96	100	96	100
Fecundity index (%)[d]	96	92	100	96	88
Fertility index (%)[e]	96	92	100	96	88
Gestation index (%)[f]	100	100	100	100	100
Gestation length (days)	22.0	21.9	21.8	21.8	22.1
No. females with liveborn pups	24	22	25	23	22
Total no. live pups					
Day 1	285	277	294	285	251
Day 4, precull	258	259	267	261	240
Day 21	145	152	168	134	148
Mean no. live pups/litter					
Day 1	11.9 (24)	12.6 (22)	11.8 (25)	12.4 (23)	11.4 (22)
Day 4, precull	10.8 (24)	11.8 (22)	10.7 (25)	11.3 (23)	10.9 (22)
Day 21	6.0 (24)	6.9 (22)	6.7 (25)	5.8 (23)	6.7 (22)
Live birth index (%)[g]	99	100	99	100	98
Viability index (%)[h]	92	94	91	91	96
Lactation index (%)[i]	78	86	90	76	85
Mean pup body weight (g)					
Day 1	6.2	5.8	6.0	5.8	5.8
Day 7	11.8	11.7	12.5	11.1	11.6
Day 14	25.2	24.7	25.5	23.4	23.0
Day 21	42.4	41.2	41.8	40.1	37.4*
Sex ratio (% males, day 1)	49	51	48	48	43

[a]Data were extracted from Study No. 380-154, Tables 26, 43, 44 and 45.

[b]Number within parentheses represents number of litters.

[c]Insemination index: No. of mated females expressed as % of no. of paired females

[d]Fecundity index: No. of pregnant females expressed as % of no. of mated females

[e]Fertility index: No. of pregnant animals expressed as % of no of paired animals

[f]Gestation index: No. of females delivering a live litter expressed as % of no. of pregnant females

[g]Live birth index: Percentage of pups born alive based on no. of total pups born

[h]Viability index: Percentage of pups alive on day 4 based on no. of pups alive on day 1

[i]Lactation index: Percentage of pups surviving 21 days based on no. of live pups on day 4 postcull

*Significantly different from control ($p \leq 0.05$)

No compound-related effects in pup physical development including pinna unfolding, incisor eruption, eye opening, pupillary reflex, or auditory response were observed at any dosage level in either sex or generation.

D. REVIEWERS' DISCUSSION/CONCLUSIONS

Test Material Analyses

On a few occasions at the lower dosage levels, the analyses for concentration and homogeneity revealed values outside ±20% of target. The reviewers considered these findings to be irrelevant for the overall interpretation of this study. Stability analyses, however, showed that the test material may not have been stable in the diet for 28 days as indicated by the analytical chemistry results. This is further supported by the overall results of the stability tests (at room temperature or deep frozen at 50, 500, and 1000 ppm) after 7 days (81%), 14 days (83%), 21 days (78%), and 26 days (84%). The reviewers concluded that the actual target dosages overall were only 82% of the intended target dosages.

Parental Toxicity

Compound-related parental toxicity was observed at 500 and 1000 ppm. It was manifested as significantly decreased body weight and/or body weight gain at 500 and 1000 ppm in both sexes and generations and significantly decreased food consumption at 1000 ppm in F_1 females only. Based on these results, the NOEL and LOEL for parental toxicity were 50 and 500 ppm, respectively.

Reproductive Toxicity

Compound-related reproductive toxicity was observed at 500 and 1000 ppm. It was manifested as significantly decreased pup body weight during the latter part of lactation in both generations at 1000 ppm and in the F_1 pups at 500 ppm. Based on these results, the NOEL and LOEL for reproductive toxicity were 50 and 500 ppm, respectively.

E. CORE CLASSIFICATION: Core Guideline Data. This study meets the requirements set forth under Guideline Series 83-4 for a two-generation reproductive toxicity study in rats.

Parental NOEL = 50 ppm
Parental LOEL = 500 ppm based on decreased body weight and/or weight gain in both sexes and generations

Reproductive NOEL = 50 ppm
Reproductive LOEL = 500 ppm based on decreased pup body weight on LD 21 in both generations

F. RISK ASSESSMENT: Not applicable